SWEET BRIAR COLLEGE
3 2449 0472417 7

Digitized by the Internet Archive
in 2010 with funding from
Lyrasis Members and Sloan Foundation

http://www.archive.org/details/determinationofm00wils

DETERMINATION OF MERCURY UPTAKE IN THE COMMON GREEN BEAN *PHASEOLUS VULGARIS* BY COLD-VAPOR ATOMIC ABSORPTION SPECTROMETRY AND SCANNING ELECTRON MICROSCOPY

A Senior Honors Thesis in the Department of Environmental Studies
Sweet Briar College

By

Kathleen M. Wilson

Defended and Approved in April 2006

David R. Orvos 5 MAY 2006
Professor David Orvos (Faculty Advisor) date

05/05/06
Professor Janet Steven date

Dr. Leo Hsu 05/04/06
Dr. Leo Hsu date

ABSTRACT

The transport of heavy metal mercury within the common green bean, *Phaseolus vulgaris* was studied in this research. The study was done with five different concentrations (blank, 0.4 mg/L, 0.74 mg/L, 1.5 mg/L and 3.7 mg/L) of mercury in deionized water (DI). Mercury was added in the form of mercuric chloride. The growth of the plants continued until beans were produced. Cold-vapor atomic absorption spectrometry (CVAAS) was used to analyze the mercury concentrations with the various plant sections, soil and water eluent. Scanning electron microscopy (SEM) was also conducted to analyze any morphological changes that occurred within the plants treatment groups. Negative effects such as chlorosis and necrosis were observed on the plants. The lowest observable effective concentration (LOEC) was the 0.74 mg/L treatment group. For the CVAAS, mercury was detected in the 0.4 mg/L treatment group. The LOEC for the SEM was the 1.5 mg/L treatment group that displayed a loss of fine root hairs and a reduced number of stomata. It appears that mercury directly affected the overall health of the plant, but the concentrations of Hg detected should not inhibit the consumption of green beans.

INTRODUCTION

Humans have always held a fascination with mercury. Being a liquid at room temperature, mercury was used for various purposes over the centuries from metallurgy to medicine. Recently people have realized the detrimental health effects that mercury can have on the human body. Being a pollutant, mercury is distributed around the world through the burning of fossil fuels and contaminates the environment by air deposition. When Hg is deposited on vegetation, it can be transported up the plant. If people consume the plant, mercury can bioaccumulate in humans leading to harmful effects. This research specifically analyzed the uptake and transport of Hg in Phaseolus vulgaris to determine the concentration within the various plant tissue sections and the soil.

Mercury

Mercury is a naturally-occurring element in the Earth's crust and is represented on the periodic table as Hg. Mercury has been known to humans for thousands of years and has been used for medicinal purposes, religious rites and recently in industrial processes[31]. However, it has been human activity that has dramatically increased the amount of mercury in the environment and its distribution around the globe.

There are several forms of mercury: elemental, inorganic and organic. These can also be known as the three stable oxidation forms of Hg (0), (I), and (II). Mercury is in the same group as zinc (Zn) and cadmium (Cd) on the periodic table, all of which are considered transitional metals. Elemental mercury, Hg (0), is the most common form; the silver liquid was often used in thermometers and is mercury's pure form[1]. Inorganic mercury, Hg (I), and Hg (II), is where mercury forms complex ions with other elements. Such examples include mercuric chloride and mercuric sulfide. Mercuric chloride is important because it was the main ingredient in pesticides

and fungicides in the 1950s[9]. Finally, there is organic mercury, with the most common form being methylmercury, Hg (CH_3). It is the most deadly and toxic form of Hg and yet it is present in the environment. Methylation of mercury is a process where bacteria transform a complex mercury compound containing an H atom to have a CH_3 attached in its place. Methylmercury poses serious health risks to humans and the environment with its main impact found in fishes. Once Hg is in the environment it is very difficult to remove and often continues on the cyclic pattern. Figure 1 shows the physical and chemical processes of mercury.

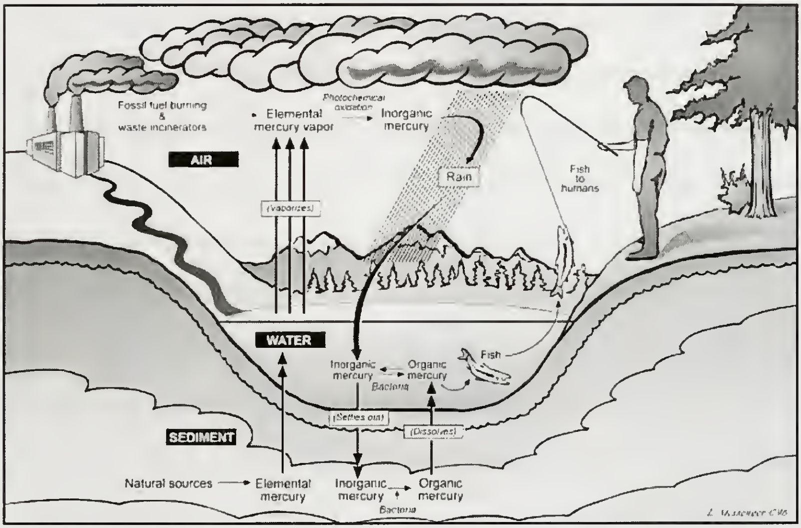


Figure 1: The chemical and physical cycle of mercury transport in the environment. Source: www.uwec.edu

Anthropogenic activities have greatly increased the amount and distribution of mercury around the globe[1]. World production of Hg was relatively constant from 1900 to 1939 with the production of 3,600 tonnes/year[1]. However, since the 1960s, mercury production has more than doubled[6]. In 1990, the total global production of mercury was 2,143 tonnes/year in 1990[1]. Then

by the year 1995, this quantity dropped to 1,912 tonnes/year due to the US EPA regulation of power plant emissions[1, 31].

Today, mercury is used in various products such as electronics, dental work and in paints. Mercury is also used in the process of gold mining to extract out the precious metal from the Earth. With its unique characteristic of being a liquid at room temperature, Hg is ideal for high thermal conductivity in industrial processes.

Air pollution is the main source of mercury in the environment today. An air pollutant is defined as any aerial substance that demonstrates harmful effects on living organisms[25]. The main environmental impact from air pollution is that all living organisms are exposed to the contaminants. The increase in mercury pollution is an anthropogenic problem. Since the industrial revolution, humans have been changing and altering the composition of the air through the burning of fossil fuels, vehicle exhaust and volatile toxic compounds.

With the discovery of coal as a main source of heat and energy, it was rapidly consumed in this country due to the diminishing wood supply[21]. Coal produces more heat than wood and was an integral part of the American Industrial Revolution. Coal also led the way for steel production in this county. Mercury is deposited throughout sedimentary rocks layers, specifically coal. The burning of coal was first recognized as a main pollutant of mercury in 1954[6]. However, mercury pollution from coal burning power plants was not regulated until 1963 with the passing of the Clean Air Act by Congress. The amount of mercury released by the combustion of coal depends on the amount initially in the coal. Studies have been conducted to determine the amount of mercury in coal. Samples of American coal contain concentrations of mercury within the range of 0.070 – 33 parts per million (ppm)[20]. Additionally, mercury concentrations detected in the air tend to be higher in the Eastern part of the United States than the Western part[16].

Deposition of mercury and increase in concentration levels are directly related to the proximity and vicinity to a coal burning power plant[15, 30, 35]. Weather patterns and conditions must also be accounted for when analyzing the distribution of mercury. Mercury levels typically increase in the spring and summer months due to the increase in rain. This increase in wet deposition may directly impact the growing season of agricultural crops with the mercury pollution at the early stage of plant growth. Of the mercury in the atmosphere, 60% is suspected to be deposited on land with a suspected 40% deposited on water[21].

Currently, global economic conditions make coal the most important fuel source for power and coal will continue to be the main source for the next three decades[12]. Even with the modern technological advances in power plants such as "scrubbers" mercury pollution is still occurring. Additionally, these "scrubbers" are only incorporated in wealthy nations and will not be implemented into third world countries for several decades[21]. Nevertheless, these scrubbers have reduced the amount of mercury released from coal burning power plants. When coal is burned the elemental Hg gets volatilized by the intense heat and escapes to the atmosphere. Once in the atmosphere, it has been documented that Hg travels great distances[1, 21]. Such an example includes the unusually high concentrations of mercury in the Arctic. Volatilized mercury readily adheres to any particulate in the air and can remain there for several decades, reaching remote locations thousands of miles from its origination.

Mercury contamination occurs in all ecosystems around the world. When the mercury particle lands in a body of water or waterway, it is suspended in the aqueous solution until it is captured by primary producers. Once at this primary trophic level, organisms in higher trophic levels consume the mercury. The major intake of mercury (Hg(II)) for humans is through the consumption of fish and seafood. The second most common form of human consumption of

mercury is through agricultural and horticultural crops[18]. Mercury is a bioaccumulator, which means that it gradually increases in concentration and can reach toxic levels as it moves within the food web[22, 27]. Once in an organism, mercury can be excreted through the feces or urine. However, excretion is dependent on the size of the dose or concentration, the form of mercury and the overall health of the organism. Therefore, by the time humans ingest the food source containing mercury, the levels can be toxic. When mercury comes in contact with land, it undergoes complex chemical and biological processes and environmental conditions. It is here that mercury adheres to the soil and becomes available for nutrient uptake by plants. Methyl mercury and Hg (II) are both reactive with cellular components and are assimilated by microorganisms[28].

Mercury can be toxic to humans depending on the age, sex and overall health of the individual. In adults, mercury poisoning has been known to cause kidney failure, central nervous system deterioration and even death. The populations most at risk for health effects from mercury poisoning are infants and the elderly. In infants, mercury can lead to neurological defects, nervous system failure and immune system deficiency. In the elderly, mercury accumulation leads to organ dysfunction and chronic toxicty[6, 24]. For these health reasons the Federal government wants to decrease mercury intake through the use of policy regulation. By the year 2010 the government plans to decrease mercury emissions to only 33% of its current production levels. Being a fossil fuel, coal is in limited quantities, but of the fossil fuels, coal is the most abundant and widely distributed source of energy[21]. This reduction will be beneficial to future generations, but the presence of mercury will always coexist with humans and our energy production.

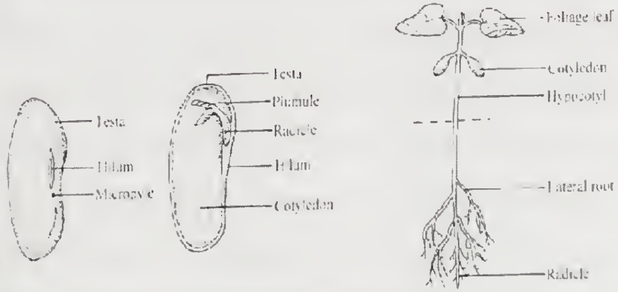
Being a pollutant all around the world, mercury is under different regulations in various countries. The nation with the strictest legislation on mercury pollution is the United States. The U.S. EPA regulates the quantity of Hg released each year as pollution from all anthropogenic sources. In the early 1990s, mercury levels were decreased due to new laws limiting mercury release from municipal waste combustors and medical waste incinerators[31]. However, under the Bush Administration, no new Hg legislation has been passed to curtail the amount of mercury produced by coal burning power plants. With the passing of the Clean Skies Act, mercury will be regulated by a "cap and trade" mechanism[31]. This same approach is used in the regulation of acid rain. Additionally, acid rain levels have increased and this regulation approach is being highly criticized by experts[16]. They also debate that this approach due to being unsuccessful for acid rain will work for mercury.

Mercury is re-introduced to terrestrial and aquatic systems through either wet deposition or dry deposition. Dry deposition is where acidic particles and gases get distributed by wind. The particles are mainly blown onto houses, buildings, cars and trees. Then through rain, these deposited particles are washed from their surfaces and re-deposited into the environment[12]. Wet deposition, the more common form of mercury pollution in the environment, gets transported back to the land and water by the water. Wherever these droplets land, contamination then occurs in the environment. If deposited on vegetation, injury can occur to the plant, such as reduction in reproduction and possibly necrosis[9].

Phaseolus vulgaris

The common green bean, *Phaseolus vulgaris (Fabaceae)* is a dicotyledonous plant, which is a flowering plant that has two embryonic seed leaves or cotyledons that appear at germination. The green beans are angiosperms which are the most important group of plants and

there are over 25,000 different species[3]. Flowering plants produce the most biomass on land and comprise nearly all of the world's main food supply[7]. Beans have a trifoliate leaf symmetry and an overall bilateral symmetry for the entire plant[3, 17, 32]. Mercury has analyzed for its detrimental effects that is cause to the various growth stage of *Phaseolus vulgaris*[2,18,23]. Additionally, Figure 2 is a schematic of bean seeds and the juvenile form of the growth stages[13].



Epigeal germination and seed structure of *Phaseolus vulgaris*

Figure 2: The growth stages of green beans. This is relevant to understand the development of the plants and the possible effects of mercury pollution can have on the growth stages[13].

Starting at the base of most plants are the roots. Roots serve as the main source of absorption of water and nutrients from the soil. In legumes, roots work in combination with nitrogen fixing bacteria serve as a nitrogen source. The stem is the main structural support of the plant. The stem also governs the position of buds and leaves and the height of the stem influences the light received by the plant. The leaves are the main photosynthesis organs of the plant[3, 7, 9, 32]. Generally in dicotyledons, there is a large leaf blade to capture light and increase photosynthesis. Stomata aid in CO_2 and water exchange in the plant. Before fruit is produced, flowers are used by the plant for reproduction. All dicot flowers contain sepals, petals, stamens

and carpels, except those that are unisexual[32]. A typical flower is a hermaphrodite, containing both sexes and beans are capable of self-fertilization. After fertilization the seed and fruit are produced. In angiosperms, the seeds are enclosed within the fruit. The fruit is usually the final growth stage of a plant, since it has reached reproduction. Beans are classified as dehiscent fruits meaning that the fruit is fleshy and the seeds rely on external carriers for dispersal.

Growing conditions of a plant may become altered from the mercury pollution and the plant may respond with abnormal growth symptoms. These symptoms are often classified in one of four categories: growth response, reproductive effects, chlorosis and necrosis[9, 20]. Growth response is difficult to identify and monitor in plants as growth in plants is dictated by respiration and photosynthesis. There are many environmental factors that can affect either of these two growth components. Such factors include light availability, soil moisture, pH and air pollution. Air pollution can affect fruit development, which directly inhibits the reproduction of the plant[28].

Chlorosis is the "bleaching" of chlorophyll in a plant. At first, the leaves begin to turn a light green, then yellow and eventually white as the chlorophyll is destroyed. Favorable conditions must exist for the plant in order for growth and development to occur. These conditions include light availability, temperature, oxygen levels and soil nutrients. If these conditions are not met, chlorosis typically appears indicating a lack in one of these areas. Necrosis is un-programmed cell death. Necrosis can occur at any developmental stage of a plant and in any plant tissue[20]. Similar to chlorosis, necrosis is the most obvious in the leaves of plants and typically chlorosis is a precursor to necrosis. Air pollutants have been shown in studies to increase the stress levels of plants to the point of necrosis[25, 34].

Air pollutant uptake by plants is a major concern and previous scientific studies have shown that *Phaseolus vulgaris* are sensitive to mercury and other heavy metals. The effect on the

overall plant depends on the general condition of the plant, its stage of development and its metabolic activity[17]. Root hairs are the main absorption and nutrient uptake pathway for plants. They are outgrowths of epidermal cells that increase the surface area underground for plants to increase their absorption rate. Heavy metals deposited in the soil can be absorbed and distributed throughout a plant[6]. Humans may then consume these plants which have translocated the heavy metal into the food chain.

The study conducted by Bontidean *et al.*[4] contested that the roots served as a barrier of mercury transport within the plant due to Hg being located in the apoplast[17]. The apoplast is the space outside of the plasma membrane within a cell[17,32]. With an increase of mercury of four hundred times in the soil, this only caused a doubling in the mercury concentration located in the leaves[4].

Mercury uptake in plants usually exhibits a hierarchical system with the highest concentrations located in the soil> roots> leaves> stems[24,28]. Mercury is highly toxic to vascular plants with a critical value of toxicity in the range of 1 to 8 ppm[28]. The major impact of this metal on plants is that it affects the proper protein and enzymatic functions within plants. With both or one of these inhibited, the plant will not develop or grow properly often resulting in the death of the plant. Due to global distribution of mercury increasing, crop damage around the world could cause adverse agricultural implications.

Plants are the bulk of food consumed around the world[7]. They serve as vital resources for nutrients, fiber and other beneficial nutrient intake. Numerous species are edible, but only about twenty species comprise 90% of our food[7,34]. Beans are derived from wild ancestors in Central America and historical evidence has shown that the Aztec culture was utilizing these wild species. With the arrival of the Spaniards, the various wild species were domesticated and

introduced to the New World. Today, beans of various species are grown all around the world and serve as the most import legume in North America and Europe[7]. Beans specifically are a vital source of protein, folic acid and vitamin C.

Purpose and Rationale of Mercury Research

Due to environmental impacts and health concerns of mercury in the ecosystem, its bioavailability in the common green bean was assessed. Mercury uptake in plants was studied to determine concentrations within various plant organ sections. Five treatment groups of mercury dosed plants were analyzed with five plants per treatment group. Additionally, water eluent and soil samples were collected and analyzed as well. Scanning electron micrograph pictures were also assessed to visually detect any morphological changes within the plant due to mercury contamination.

The first objective of this project was to determine if mercury was translocated in a bean plant and where the highest concentration occurs. Particular interest was in the bean pod, the section in which humans consume. Previous studies have analyzed the mercury content in shoots and roots (above ground and below ground) and particular interest was taken in analyzing the plant organs independently[2,18]. The second objective of this research was to determine if there were any morphological changes that occurred within the bean plants due to expose to harmful mercury concentrations.

METHODOLOGY

Experimental Design

The seeds of *Phaseolus vulgaris* were randomly selected from purchased seed packets and were planted in Peat Pots® containing purchased organic potting soil. Each Peat Pot® contained 54 grams (wet weight) of soil. The seeds were planted 3 cm deep into the pots and the pots were kept on trays. The trays were rotated every two days within the incubator for equal light and growing environment. A 14:10 light/dark cycle was used to mimic the early summer growing conditions. The incubator was set at the temperature of 25ºC with no temperature variation. The plants were watered daily with 10 mL of deionized water (DI). Using DI water reduces the interference from magnesium and copper with the mercury, which are commonly found in tap water. Gradually over the extended period of the experiment the amount of DI water was increased to 30 mL per plant to accommodate plant growth. Also, a beaker of DI water was set in the growth chamber to increase atmospheric moisture for the plants. Water eluent and pH were monitored for each plant throughout the experiment's duration. The pH was measured by strips every week to determine the pH range for the soil.

When the plants were approximately 1 cm above the top of the soil in the Peat Pot®, they were randomly selected and dosed with the following mercury concentrations; 0.0 mg/L, 0.4 mg/L, 0.74 mg/L, 1.5 mg/L, and 3.7 mg/L. The concentrations were made from mercuric chloride because $HgCl_2$ is a main chemical used in pesticides. After adding the mercury, the plants were not watered for 24 hours to ensure absorption. The mercury concentrations were made up the day of dosing to ensure the accurate amount. A randomized block design was used with all of the plants on the two trays. The project lasted for 12 weeks.

Atomic Absorption Spectrometry Sample Preparation

Prior to analysis, each plant was photographed and then each of the plant organs was photographed and weighed. Data were also collected including number of leaves, number of leaves showing signs of chlorosis, number of beans, length of stem and length of roots. In order for a leaf to be included in the chlorotic data count was that if there were any signs of chlorosis on the leaf, it was deemed as such. Then the leaves, beans, and stem were placed in a labeled brown paper bag to dry over night. The soil and roots were placed on labeled sheets of wax paper to dry over night. After 24 hours each of the plant tissues were reweighed to the dry weight for each component[14]. Finally, each of these components were placed in pre-cleaned and pre-labeled 125 mL digestion vessels (Environmental Express®).

The water eluent samples were collected on the fifth day of watering for the plants. The Peat Pots were lifted up on the tray by catchment vials which were emptied into the digestion vessels. The volume was recorded for each treatment group of plant and 1 mL of Omni-pure nitric acid was added to each vessel. This was done to acidify the water and to keep the potential mercury in the vial from volatilizing. The water eluent samples were kept in the refrigerator until analysis. Collection of the water eluent occurred after the dosing of the various Hg concentrations.

SEM Sample Preparation

Prior to analysis, each sample was fixed, dried, and sputter coated[3]. Five samples parts, leaves, stems, roots were prepared for each plant. Every sample was fixed in formalin overnight before undergoing the five-step graded ethanol series. Sublimation in Peldri® is the final step in this process. Next, the plant tissue sections were mounted on stubs using double-sided carbon tape. The samples were then sputter coated in gold-platinum and analyzed with the SEM[5,11].

AAS Sample Extraction

For the sample extraction process the US EPA method 3050 B and 7417 standard methods were used for the acid digestion analysis. A Hot Block® (Environmental Express) was used for heating the samples to 105°C (95°C inside the vessels) and the samples were elevated with the lifters[2]. Plastic watch glasses were also used to ensure the condensation of the vapors. The Hot Block® holds 25 samples at a time, therefore the samples were digested over a period of three days. Additionally, standard reference material (SRM) from the National Institute of Science and Technology of tomato leaves and acid blanks were incorporated in this study to test the accuracy of the digestion process. All of these samples were analyzed under the same conditions as the plant tissues samples. For the soil samples, prior to analysis, approximately 1 gram of dry weight the soil was weighed and placed in the Environmental Express® digestion vessels.

AAS Sample Analysis

To analyze the mercury in the plant tissue samples a PerkinElmer AAnalyst 800 atomic absorption spectrometer with the cold-vapor technique was used (CVAAS). First, several air blanks and water (HPLC grade) were run to clean the system. Next mercury standards were analyzed to calibrate the instrument and for interpretation of the data. Then the plant tissue samples were analyzed on the AAS. Due to the quantity of samples, the analysis process was extended over three days and on each day the reductant of sodium borohydride (3% v/v) was freshly made. Additionally, water blanks were analyzed after every ten samples to check for any possible carry-over contamination on the instrument. Specifically, after each soil sample, the injection tube for the cold-vapor was rinsed with methanol to reduce possible contamination

between samples due to high effervescence. The data were saved and exported in a tab-delimited form to Microsoft Excel™ to guarantee accurate data transfer.

RESULTS

Determination of mercury in plant tissues

The concentrations of mercury were determined by cold-vapor atomic absorption spectroscopy analysis. In order to determine the mercury levels, a standard reference material was analyzed under the same conditions as all the samples on the AAS. In addition, a standard reference curve was plotted using the mass, (ng) vs. the absorbance of the standards (Figure 3). The R^2 value was 0.998. An absorbance reading for each sample was determined by the instrument and calculations were performed to determine the original mercury content (Figure 4 to 8). Using the standard curve equation, concentrations of mercury were calculated for all of the plant, soil, SRMs, blanks and water eluent samples. The plants receiving the highest dosing of mercury had the highest concentrations in the soil (Figure 7 and 8). For the beans, the highest concentration of mercury was detected in the 0.4 mg/L treatment group (Figure 9). For the remaining plant organs, Figures 9 to 12 show the detected mercury concentration. Data was collected on the number of leaves, number of beans, length of stem, length of roots and the mass of all these plant organs (Tables 1 to 5). The water eluent collection volumes (Table 6) and the mercury content in the water eluent shows the anticipated decreased trend as the project continued (Figure 14).

Morphological changes in plant tissues

To determine morphological changes in plants due to mercury contamination, scanning electron microscopy was used. Micrographs were taken for each plant tissue sample. The stems displayed no morphological changes and are therefore not included. The roots were the first plant tissue to show morphological differences. In the blank, 0.4 mg/L, and 0.74 mg/L fine root hairs

are present. In the 1.5 mg/L concentration, the absence of fine root hairs is visually notable. The 3.7 mg/L concentration also displays the lack of hairs on the secondary roots (Figures 14 and 15). The leaves also demonstrate morphological changes. Again, the 1.5 mg/L concentration was the first with reduced number of stomata present. The 3.7 mg/L also had similar results of reduced number of stomata (Figures 16 and 17).

DISCUSSION

Concentrations of mercury were found in the various plant tissues, concluding that by wet deposition, mercury uptake and distribution occurs in the common green bean. These data support the previous scientific studies with similar findings. The research conducted by Smart[39] found that when mercury was directly applied to some parts of the plants, it was translocated to other various sections within them. Smart[39] also suggested with this study, Smart concluded that the mercury concentrations would be within the part per billion range. This statement was later confirmed by Gracey and Stewart[38] and Gowen *et al.*[40]. Recently, Mor *et al.*[23] found that in *Phaseolus vulgaris* in the presence of gibberellins (GA_3) the overall mercury toxicity level was reduced in the seedlings. The most recent study conducted by Bontidean *et al.*[4] found that there is a direct correlation to the quantity of mercury in the soil and the concentration detected in the leaves.

The lowest observed effective concentration (LOEC) of mercury to show plant response was the 0.74 mg/L. Through the CVAAS analysis, mercury was detected in all treatment groups and within all plant tissue sections. This result was unexpected because the blank treatment group did not receive a dosing of mercury, yet it was detected in the plants. One possible explanation for this result is that the other treatment groups within the incubator volatilized the mercury through respiration. With the mercury in the closed system of the incubator, it was then reabsorbed by all the plants. This hypothesis has yet to be supported by scientific research.

The LOEC for the roots and leaves that was viewed with the SEM to show morphological changes was 1.5 mg/L. Such morphological chances include loss of secondary fine root hairs and a decrease in the number of stomata on the leaf surface. The absence of fine root hairs could be caused from the sonication process to remove dirt or brittleness caused by exposure to mercury.

In Mor *et al.*[23] study which found that at the concentration of 0.1 mM of mercury roots were in a brittle state. This result probably was caused by the absorption of nutrients and water by the roots, thus being directly exposed to mercury. The reduced number of stomata on the leaf surface may be attributed to mercury inhibiting the development and growth of the plant. With a reduction in stomata, respiration and evaporation of water from the plant directly affects the survival of the organism[15]. This is a correlation to the ecological energy equation: $E=M+G+R$ where, E is energy, M is maintance, G is growth and R is reproduction. Due to receiving a high dose of mercury, these plants were balancing their energy equation to equilibrate the exposure to a heavy metal.

Another possible biological example of the bean plants resisting this heavy metal toxicity was that at the highest concentrations of 1.5 mg/L and 3.7 mg/L, the leaves fell off the plants. This could be a process of amelioration[9,25]. Amelioration is where plants absorb the toxins and then distribute the toxins to the tissues. This distribution localizes and dilutes the toxins within the plant. Possibly, the bean plants were ameliorating the mercury to the leaves, and then dropping the leaves as a method of elimination[9].

The plants that received the highest dose of mercury, 1.5 mg/L and 3.7 mg/L produced a minimal number of beans and the highest concentration of mercury was detected in the soil. For the 0.74 mg/L treatment group, the plants retained the most mercury in the leaves. The high concentration in the leaves could be caused by the possible amelioration process of mercury from the plants. For the 0.4 mg/L treatment group these plants produced twelve beans total and again had the highest detected mercury in the leaves, thus supporting the possible amelioration process. For the blank treatment group, the highest concentration of mercury was detected again in the leaves and a total of eleven bean pods were produced.

Both of the values that were the first to show observable effects on the bean plants are below the US EPA average concentration found in the United States. Mercury was still detected in the plant treatment groups and could have possible health effects if consumed. However, if the rate of mercury released into the environment increases, then concentrations may approach the levels of this study. This increase of mercury levels could have global agricultural implications. With the possible increase and global distribution of mercury, crops everywhere are at risk of bioaccumulating mercury and transporting it within the food web.

One such concern is the economic development of China and their degradation of the environment. With their population increase and their advancement to a global powerhouse, the Chinese government is desperately trying to meet various demands[30]. One such demand is energy. China produces nearly 50% of the world's mercury pollution. Due this increase, mercury emissions from China have a global impact and ramifications on the rest of world.

There have been several scientific advancements in the study and monitoring of mercury in the environment. From a discussion with an US EPA scientist who is studying mercury, the government has developed an unmanned plane to monitor Hg concentrations and distribution in the atmosphere over the continental United States (personal communication). With this new technology, the EPA will have better information and data to fully understand the movement of mercury in the atmosphere and its possible site of deposition in conjunction with weather patterns.

The importance and relevance of this research is applicable to the environment and the community garden on Sweet Briar's campus. In the fall of 2004, water samples were collected from the automatic air deposition meter located on campus by the observatory. In this study, mercury concentrations were detected. By identifying that mercury is in the immediate area of

Sweet Briar, this aided in the significance and importance of this project. However, soil samples and other plant samples should be tested to determine mercury concentration in the terrestrial environment of Sweet Briar. In conjunction with these tests, soil samples of the organic potting soil and the bean seeds should be tested for their mercury content prior to planting.

However, all of the LOEC levels, 0.74 mg/L and 1.5 mg/L are below the US EPA's average concentrations in the environment. Therefore, the possible toxicity of mercury on Sweet Briar's campus having detrimental health effects on individuals is minimal. Nevertheless, one must be aware of the potential harm from long term exposure to mercury and its effects on the human body. The human body produces metallothioneins to bind up the heavy metal radicals in the body before they cause serious harm. However, the body only produces a limited number of metallothioneins to act as binding sites. When all of these are full, the metal is thus allowed to be "free" in the body and cause its detrimental health effects.

Another environmental implication that arises from this study was in the analysis of the water eluent. Throughout the study, water eluent samples were collected and analyzed by the CVAAS. The overall general trend in the water eluent was as expected, with the highest concentrations occurring after the dosing and then decreased throughout the duration of the project. Then as the project progressed, the mercury concentrations within the water samples became more dilute. This result is as anticipated, yet it provides crucial information. If mercury is transported from the soil to the water eluent, this is deleterious to all ground water systems. The probability of mercury contamination of the ground water is a major concern for all cities around the world.

CONCLUSION

The mercury concentrations were detected in all plant tissue sections of all the treatment groups. The highest detected concentration for the blank treatment group was 4.030 mg/Kg located in the leaves. For the 0.04 mg/L treatment group, the highest concentration was 10.856 mg/Kg also located in the leaves. The 0.74 mg/L treatment group had the highest detected concentration of mercury in the leaves at 22.064 mg/Kg. For the 1.5 mg/L treatment group, the highest mercury concentration was 12.420 mg/Kg located in the soil. The soil also contained the highest concentration of mercury for the 3.7 mg/L at 16.864 mg/Kg.

Specifically for the beans, the plant organ most consumed by humans, the mercury levels are as follows: Blank = 2.5 mg/Kg, 0.04 mg/L = 6.7 mg/Kg, 0.74 mg/L = 0.6 mg/Kg, 1.5 mg/L = 1.0 mg/Kg, and 3.7 mg/L = 1.3 mg/Kg. The lower values for the 0.74, 1.5 and 3.7 mg/L treatment groups could be due to the ecology energy equation of $E= M+G+R$. Tables 1 to 5, show the data for these plants and which ones did not produce beans.

Table 6 shows the water eluent samples collected for each of the treatment groups over the duration of the project. In analyzing the mercury concentrations within the eluent samples, the results were similar to those of previous studies, where the mercury levels decreased over time (Figure 14).

Works Cited

1. Adriano, D. C. Trace Elements in the Terrestrial Environment. New York: Springer-Verlag, 1986.

2. Bache, Carl, and Walter Gutenmann. "Mercury and Methylmercury Content of Agricultural Crops Grown on Soils Treated with various Mercury Compounds." Journal of Agricultural Food Chemistry 21.4 (1973): 607.

3. Bell, Peter. Green Plants: Their Origin and Diversity. Oregon: Dioscorides Press, 1992.

4. Bontidean, Ibolya, and Alessia Mortari. "Biosensors for Detection of Mercury in Contaminated Soils." Environmental Pollution 131 (2004): 255.

5. Bray, D., J. Bagu, and P. Koegler. "Comparison of Hexamethyldisilazane (HMDS), Peldri II, and Critical-Point Drying Methods for Scanning Electron Microscopy of Biological Specimens." Microscopy Research and Technique 26 (1993): 489.

6. Chaignon, V., and P. Hinsinger. "Heavy Metals in the Environment." Journal of Environmental Quality 32 (2003): 824.

7. Chrispeels, Maarten, and David Sadava. Plants, Food and People. San Francisco: W.H. Freeman and Company, 1977.

8. Falandysz, Jerzy. "Mercury Content and its Bioconcentration Factors in Wild Mushrooms at Lutka and Morag, Northeastern Poland." Journal of Agricultural Food Chemistry 51 (2003): 2832.

9. Fitter, Alastair, and Robert Hay. Environmental Physiology of Plants. third ed. New York: Academic Press, 2002.

10. Gray, Netta, and Harry Fuller. "Effects of Mercury Vapor upon Seed Germination." American Journal of Botany 29.6 (1942): 456.

11. Hall, J. L., and C. Hawes, eds. Electron Microscopy of Plant Cells. New York: Academic Press, 1991.

12. Hanisch, Carola. "Where is Mercury Deposition Coming from?" Environmental Science and Technology 32.7 (1998): 176A.

13. Hickey, Michael, and Clive King. The Cambridge Illustrated Glossary of Botanical Terms. Cambridge UK: Cambridge University Press, 2000.

14. Kalra, Yash, ed. Handbook of Reference Methods for Plant Analysis. New York: CRC Press, 1998.

15. Krishna, M. V., D. Karunasagar, and J. Arunachalam. "Study of Mercury Pollution Near a Thermometer Factory using Lichens and Mosses." Environmental Pollution 124 (2003): 357.

16. www.epa.gov. “Mercury” and “The Mercury Report to Congress”, 1997.

17. Lack, A. J., and D. E. Evans. Plant Biology. Oxford UK: BIOS Scientific Publishers Limited, 2001.

18. Leonard, Todd, et al. "Mercury and Plants in Contaminated Soils:1. Uptake, Partitioning, and Emission to the Atmosphere." Environmental Toxicology and Chemistry 17.10 (1998)

19. ---. "Mercury and Plants in Contaminated Soils:2. Environmental and Physiological Factors Governing Mercury Flux to the Atmosphere." Environmental Toxicology and Chemistry 17.10 (1998)

20. Lott, John N. A. "A Scanning Electron Microscope Study of Green Plants." C.V. Mosby Company, 1976.

21. Lutter, Randall, and Elisabeth Irwin. "Mercury in the Environment: A Volatile Problem." Environment 44.9 (2002)

22. Manahan, Stanley. Environmental Chemistry. 7th ed. New York: Lewis Publishers, 2000.

23. Mor, Ilza, Sonal Gokani, and Sumitra Chanda. "Effect of Mercury Toxicity on Hypocotyl Elongation and Cel Wall Loosing in Phaseolus Seedlings." Journal of Plant Nutrition 25.4 (2002): 843.

24. Morel, Francois, Anne Kraepiel, and Marc Amyot. "The Chemical Cycle and Bioaccumulation of Mercury." Annual Review of Ecology and Systematics 29 (1998): 543.

25. Mudd, J. Brian, and T. T. Kozlowski, eds. Responses of Plants to Air Pollution. New York: Academic Press, 1975.

26. Navarro-Alarcon, Miguel. "Determination of Mercury in Crops by Cold Vapor Atomic Absorption Spectrometry After Microwave Dissolution." Journal of Agricultural Food Chemistry 39 (1991): 2223.

27. Newman, Michael, and Michael Unger. Fundamentals of Ecotoxicology. 2nd ed. New York: Lewis Publishers, 2003.

28. Orcutt, David, and Erik Nilsen. Physiology of Plants Under Stress, Soil and Biotic Factors. New York: John Wiley and Sons, Inc., 2000.

29. Rose, Jane, Michael Hutcheson, and Carol West. "Fish Mercury in Massachusetts, USA Lakes." Environmental Toxicology and Chemistry 18.7 (1999): 1370.

30. Seigneur, Christian. "Global Source Attribution for Mercury Deposition in the United States." Environmental Science and Technology 38 (2004): 555.

31. Selin, Noelle. "Mercury Rising: Is Global Action Neede to Protect Human Health and the Environment?" Environment 47.1 (2005)

32. Taiz, Lincoln, and Eduardo Zeiger. Plant Physiology. Third ed. Massachusetts: Sinauer Associates, Inc., Publishers, 2002.

33. Treshow, Michael, ed. . New York: John Wiley and Sons, 1984.

34. ---. Environment and Plant Response. New York: McGraw-Hill Inc, 1970.

35. Vanarsdale, Alan. "Patterns of Mercury Deposition and Concentrations in Northeastern North America (1996-2002)." Ecotoxicology 14 (2005)

36. Wilkinson, J. M., J. Hill, and Phillips, C. J. C. "The Accumulation of Potentially-Toxic Metals by Grazing Ruminants." Proceedings of the Nutrition Society 62 (2003): 267.

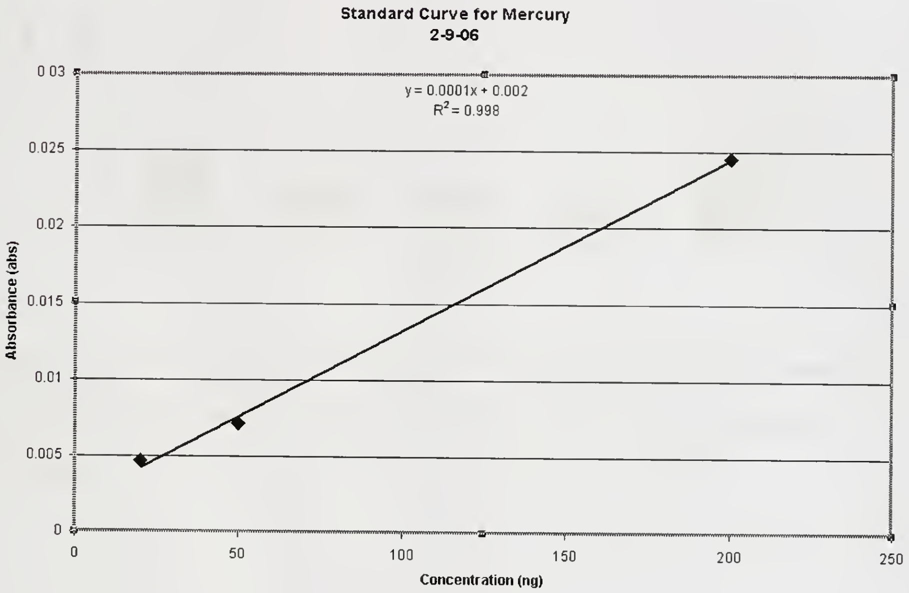
37. www.usda.gov. “Green Beans, *Phaseolus vulgaris*” species information. 2005

38. Gracey, H. I., Stewart, J. W. “In Proc Intl Conf on Land Waste Mgmt.” 97-103. Ontario Canada

39. Smart, N. A. “Mercury in Plants.” Journal of Science Food Agricultural 15 (1964): 102.

40. Gowen, J. A., Wiersma, G. B., Tai, H.“Uptake and Translocation of Mercury in Plants” Pesticides Monitoring Journal. 10 (1976) 111.

Figure 3: A plot of mercury standards and the line of best fit ($R^2 = 0.998$) and the equation of the line to calculate the mercury concentration for the plant, soil, blanks, SRMs and water eluent samples.



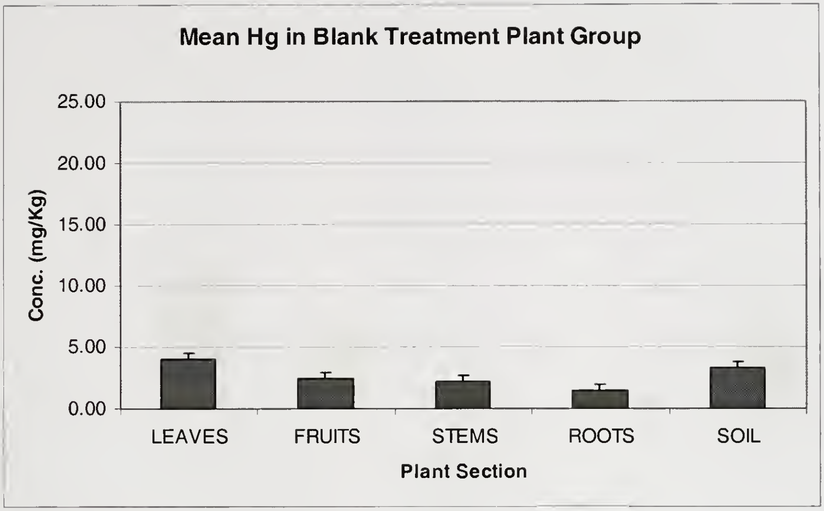


Figure 4: Various concentrations of mercury detected by the AAS of the blank treatment group. The bars on this graph represent 1 standard deviation unit. For this treatment group there were five plants.

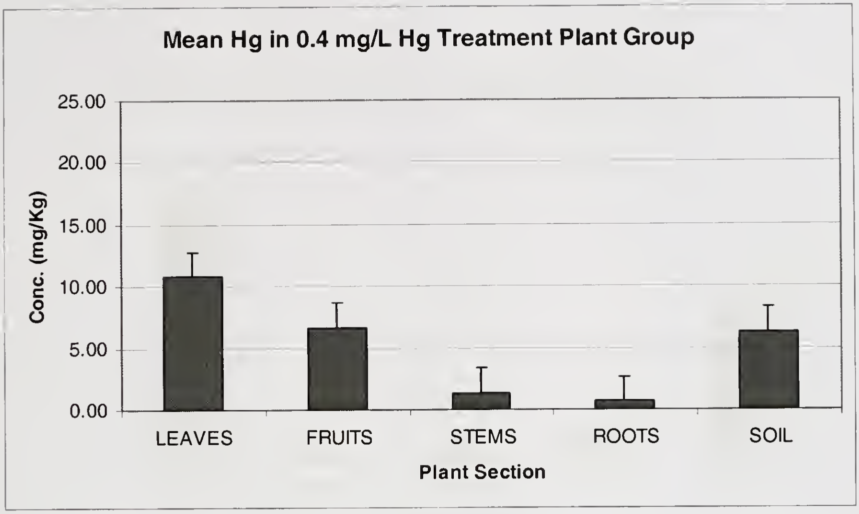


Figure 5: Various concentrations of mercury detected by the AAS of the 0.4 mg/L treatment group. The bars represent 1 standard deviation unit. For this treatment group there were five plants.

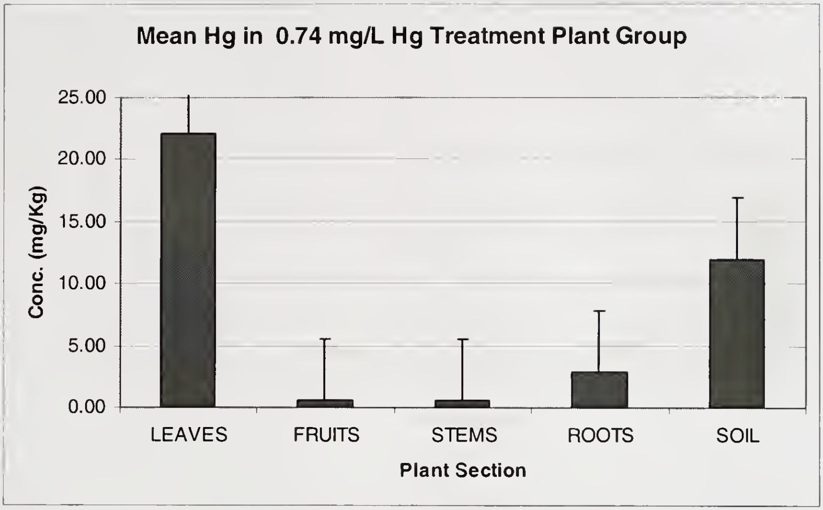


Figure 6: Various concentrations of mercury detected by the AAS of the 0.74 mg/L treatment group. The bars represent 1 standard deviation unit. For this treatment group there were five plants.

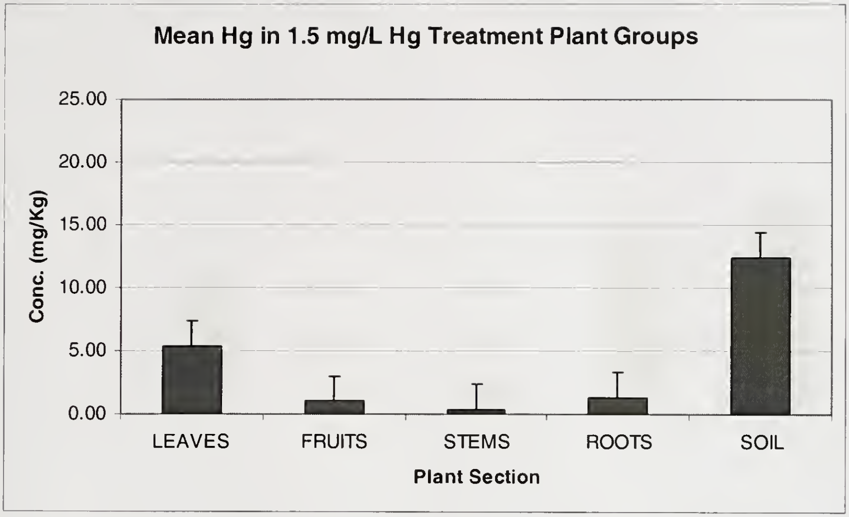


Figure 7: Various concentrations of mercury detected by the AAS of the 1.5 mg/L treatment group. The bars represent 1 standard deviation unit. For this treatment group there were four plants. One individual was lost during the duration of the research.

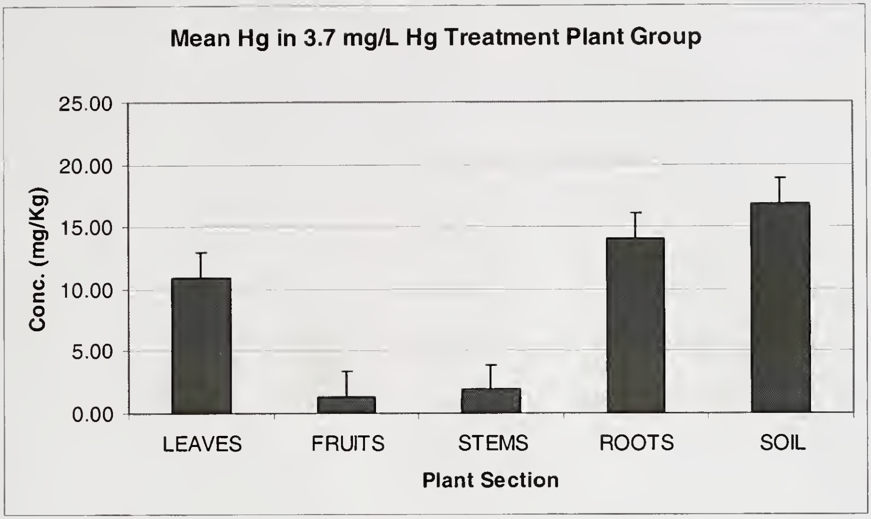


Figure 8: Various concentrations of mercury detected by the AAS of the 3.7 mg/L treatment group. The bars represent 1 standard deviation unit. For this treatment group there were five plants.

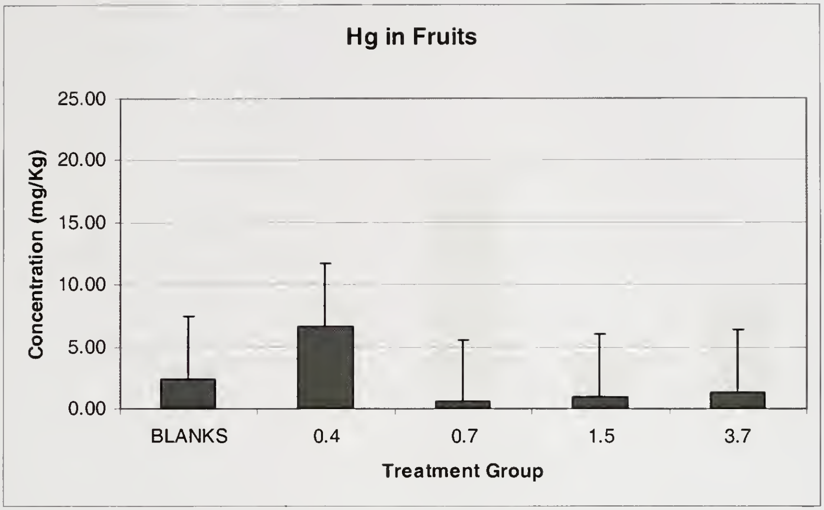


Figure 9: Average concentration of mercury in the beans in the various treatment groups. The bars represent 1 standard deviation unit.

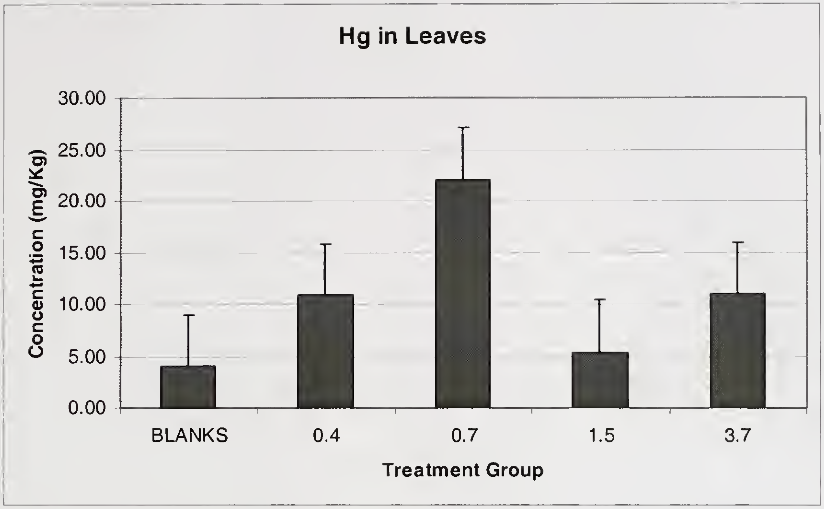


Figure 10: Average concentration of mercury in the leaves in the various treatment groups. The bars represent 1 standard deviation unit.

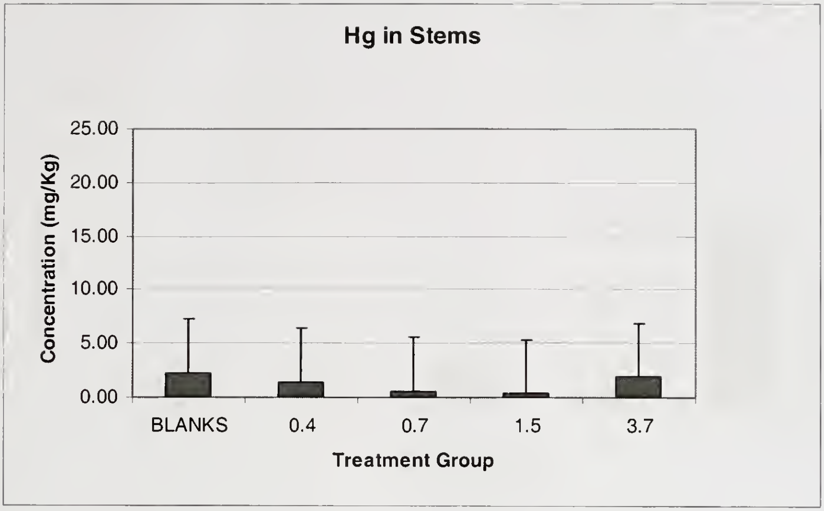


Figure 11: Average concentration of mercury in the stems in the various treatment groups. The bars represent 1 standard deviation.

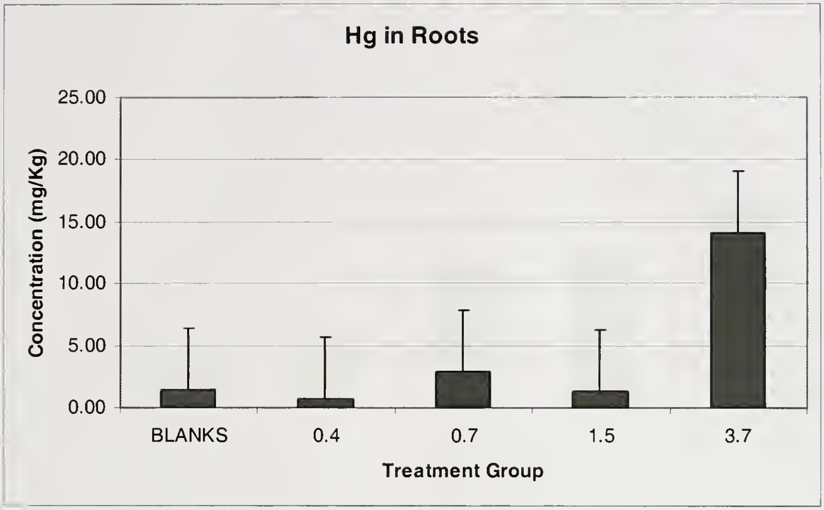


Figure 12: Average concentration of mercury in the roots in the various treatment groups. The bars represent 1 standard deviation unit.

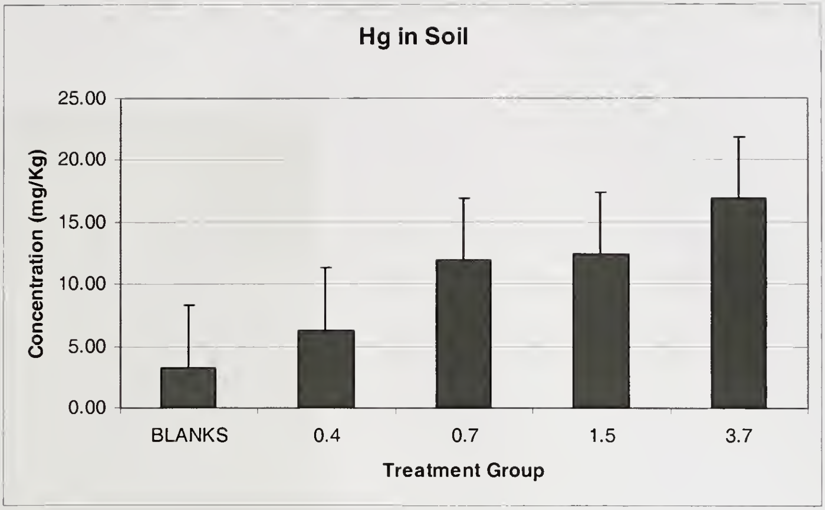


Figure 13: Average concentration of mercury in the soil in the various treatment groups. The bars represent 1 standard deviation unit.

Figure 14: SEM micrograph of a blank (control) root depicting the fine root hairs, the white structures on a secondary root.

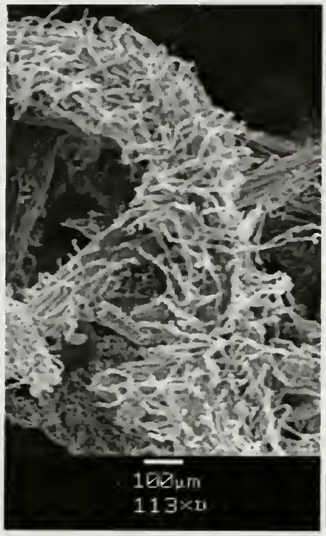


Figure 15: SEM micrograph of the 1.5 mg/L root demonstrating the absence of fine root hairs on the least observable effective concentration (LOEC) concentration of 1.5 mg/L. Note that in comparison to Figure 9, there are very few white structures on a main root in this micrograph.

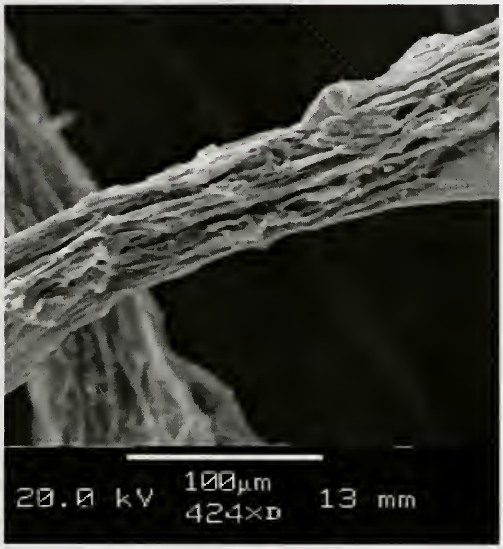


Figure 16: SEM micrograph of a blank (control) leaf visually showing stomata. They are their regular circular shape with an opening for gas and water exchange for the plant. It was easy to identify more than three stomata per viewing screen on the SEM.

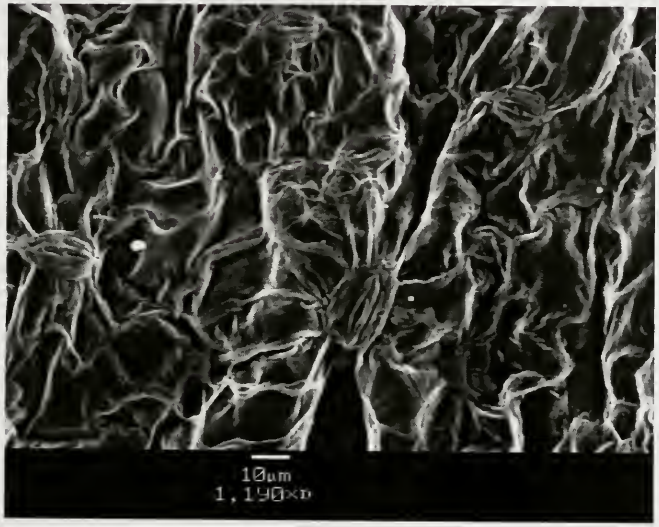


Figure 17: SEM micrograph of 1.5 mg/L leaf demonstrating the reduced number of stomata. It was difficult to identify more than three stomata per viewing screen on the SEM. With a reduced number of stomata, the overall growth of the plant could be inhibited.

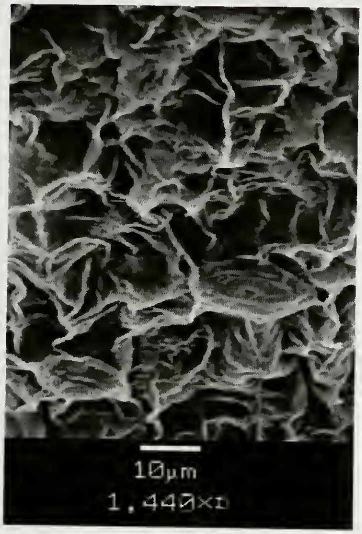


Table 1: Data collected prior to analysis for the blank Hg treatment group prior to AAS analysis. The units for mass are milligrams (mg). The units for length are centimeters (cm).

BLANK

A	# of leaves	17
	# of beans	3
	# of flowers	0
	# of chlorotic	0
	mass of leaves	3.4910
	mass of stem	2.1146
	mass of beans	1.9370
	mass of soil	85.41
	mass of roots	3.5508
	length of stem	37.0
	length of roots	15.0

B	# of leaves	6
	# of beans	2
	# of flowers	0
	# of chlorotic	6
	mass of leaves	1.1603
	mass of stem	1.7378
	mass of beans	3.4458
	mass of soil	96.41
	mass of roots	3.7276
	length of stem	40.5
	length of roots	18.0

C	# of leaves	10
	# of beans	2
	# of flowers	0
	# of chlorotic	4
	mass of leaves	2.6614
	mass of stem	2.0759
	mass of beans	4.9693
	mass of soil	80.89
	mass of roots	2.5817
	length of stem	35.0
	length of roots	13.0

D	# of leaves	14
	# of beans	2
	# of flowers	0
	# of chlorotic	7
	mass of leaves	2.6873
	mass of stem	1.8651
	mass of beans	2.1507
	mass of soil	102.73
	mass of roots	2.6940
	length of stem	36.0
	length of roots	12.0

E	# of leaves	9
	# of beans	2
	# of flowers	0
	# of chlorotic	5
	mass of leaves	1.3811
	mass of stem	1.5851
	mass of beans	1.8077
	mass of soil	96.33
	mass of roots	3.4667
	length of stem	35.5
	length of roots	13.0

Table 2: Data collected prior to analysis for the 0.4 mg/L Hg treatment group prior to AAS analysis. The units for mass are milligrams (mg). The units for length are centimeters (cm).

0.4 mg/L

A	# of leaves	8	**B**	# of leaves	11	**C**	# of leaves	20
	# of beans	1		# of beans	1		# of beans	5
	# of flowers	0		# of flowers	0		# of flowers	0
	# of chlorotic	8		# of chlorotic	9		# of chlorotic	4
	mass of leaves	2.4315		mass of leaves	2.1503		mass of leaves	3.7211
	mass of stem	1.6577		mass of stem	1.0470		mass of stem	2.8765
	mass of beans	3.1838		mass of beans	2.8816		mass of beans	3.3140
	mass of soil	77.63		mass of soil	64.46		mass of soil	94.43
	mass of roots	4.5630		mass of roots	5.9742		mass of roots	5.5992
	length of stem	37.0		length of stem	50.0		length of stem	70.0
	length of roots	12.0		length of roots	25.0		length of roots	13.0

D	# of leaves	14	**E**	# of leaves	8
	# of beans	4		# of beans	1
	# of flowers	0		# of flowers	0
	# of chlorotic	10		# of chlorotic	4
	mass of leaves	1.9589		mass of leaves	1.8993
	mass of stem	1.2679		mass of stem	1.5596
	mass of beans	0.5899		mass of beans	0.7039
	mass of soil	98.46		mass of soil	97.73
	mass of roots	3.2507		mass of roots	2.9996
	length of stem	33.0		length of stem	31.0
	length of roots	11.0		length of roots	14.5

Table 3: Data collected prior to analysis for the 0.74 mg/L Hg treatment group prior to AAS analysis. The units for mass are milligrams (mg). The units for length are centimeters (cm).

0.74 mg/L

A	# of leaves	14
	# of beans	2
	# of flowers	0
	# of chlorotic	7
	mass of leaves	2.3654
	mass of stem	1.5468
	mass of beans	0.9540
	mass of soil	98.95
	mass of roots	4.8040
	length of stem	34.0
	length of roots	11.0

B	# of leaves	21
	# of beans	0
	# of flowers	0
	# of chlorotic	8
	mass of leaves	1.9517
	mass of stem	1.653
	mass of beans	NA
	mass of soil	108.25
	mass of roots	4.492
	length of stem	51.0
	length of roots	11.5

C	# of leaves	20
	# of beans	0
	# of flowers	0
	# of chlorotic	12
	mass of leaves	3.4167
	mass of stem	2.4913
	mass of beans	NA
	mass of soil	70.61
	mass of roots	5.2240
	length of stem	31.0
	length of roots	10.5

D	# of leaves	18
	# of beans	0
	# of flowers	0
	# of chlorotic	7
	mass of leaves	0.7408
	mass of stem	0.9874
	mass of beans	NA
	mass of soil	114.45
	mass of roots	1.1510
	length of stem	17.0
	length of roots	28.0

E	# of leaves	8
	# of beans	1
	# of flowers	0
	# of chlorotic	3
	mass of leaves	0.9288
	mass of stem	1.3361
	mass of beans	1.6406
	mass of soil	111.79
	mass of roots	2.7113
	length of stem	33.0
	length of roots	10.0

Table 4: Data collected prior to analysis for the 1.5 mg/L Hg treatment group prior to AAS analysis. The units for mass are milligrams (mg). The units for length are centimeters (cm).

1.5 mg/L

A	# of leaves	0	**B**	# of leaves	20
	# of beans	1		# of beans	0
	# of flowers	0		# of flowers	6
	# of chlorotic	0		# of chlorotic	10
	mass of leaves	NA		mass of leaves	4.4010
	mass of stem	2.3564		mass of stem	2.9146
	mass of beans	3.671		mass of *flowers*	0.3665
	mass of soil	99.08		mass of soil	58.67
	mass of roots	5.8981		mass of roots	11.2294
	length of stem	41.0		length of stem	40.0
	length of roots	12.0		length of roots	10.0

C	# of leaves	4	**D**	# of leaves	NA
	# of beans	1		# of beans	3
	# of flowers	0		# of flowers	NA
	# of chlorotic	4		# of chlorotic	NA
	mass of leaves	1.5707		mass of leaves	NA
	mass of stem	1.5627		mass of stem	1.3729
	mass of beans	3.3537		mass of beans	1.7866
	mass of soil	74.34		mass of soil	107.31
	mass of roots	5.8663		mass of roots	3.5715
	length of stem	35.0		length of stem	36.0
	length of roots	12.0		length of roots	12.0

Table 5: Data collected prior to analysis for the 3.7 mg/L Hg treatment group prior to AAS analysis. The units for mass are milligrams (mg). The units for length are centimeters (cm).

3.7 mg/L

A	# of leaves	6	**B**	# of leaves	14	**C**	# of leaves	11
	# of beans	1		# of beans	3		# of beans	4
	# of flowers	0		# of flowers	0		# of flowers	0
	# of chlorotic	6		# of chlorotic	7		# of chlorotic	7
	mass of leaves	1.2141		mass of leaves	2.7901		mass of leaves	3.0091
	mass of stem	1.3710		mass of stem	2.1404		mass of stem	2.0366
	mass of beans	3.0744		mass of beans	2.5425		mass of beans	1.4353
	mass of soil	91.49		mass of soil	98.40		mass of soil	79.01
	mass of roots	5.0162		mass of roots	4.8905		mass of roots	4.6300
	length of stem	38.5		length of stem	50.000		length of stem	22.0
	length of roots	15.000		length of roots	13.000		length of roots	12.0

D	# of leaves	17	**E**	# of leaves	20
	# of beans	0		# of beans	0
	# of flowers	0		# of flowers	0
	# of chlorotic	10		# of chlorotic	12
	mass of leaves	1.6583		mass of leaves	3.0264
	mass of stem	1.2849		mass of stem	1.6720
	mass of beans	NA		mass of beans	NA
	mass of soil	89.94		mass of soil	89.30
	mass of roots	1.7902		mass of roots	3.2750
	length of stem	29.0		length of stem	40.0
	length of roots	10.0		length of roots	14.0

Table 6: Water eluent samples collected for each of the treatment groups over the duration of the project. The volume units are milliliters (mL). The sample units are mg/L.

Water Eluent			
10/26/2005		11/18/2005	
Sample	Vol.Collect	Sample	Vol.Collect
blank	5.0	blank	20.0
0.4	9.0	0.4	7.0
0.7	11.0	0.7	25.0
1.5	18.0	1.5	20.0
3.7	10.0	3.7	29.0
10/28/2005		11/27/2005	
blank	10.0	blank	9.0
0.4	11.0	0.4	0.0
0.7	1.00	0.7	10.0
1.5	13.0	1.5	5
3.7	9.0	3.7	10.0
11/2/2005		12/2/2005	
blank	30.0	blank	20.0
0.4	22.0	0.4	5
0.7	30.0	0.7	10.0
1.5	39.0	1.5	19.0
3.7	30.0	3.7	21.0
11/8/2005		12/7/2005	
blank	28.0	blank	20.0
0.4	20.0	0.4	5
0.7	27.0	0.7	10.0
1.5	26.0	1.5	19.0
3.7	21.0	3.7	12.0
11/13/2005		12/12/2005	
blank	21.0	blank	9.0
0.4	29.0	0.4	0.0
0.7	18.0	0.7	7.0
1.5	20.0	1.5	5
3.7	20.0	3.7	0.0

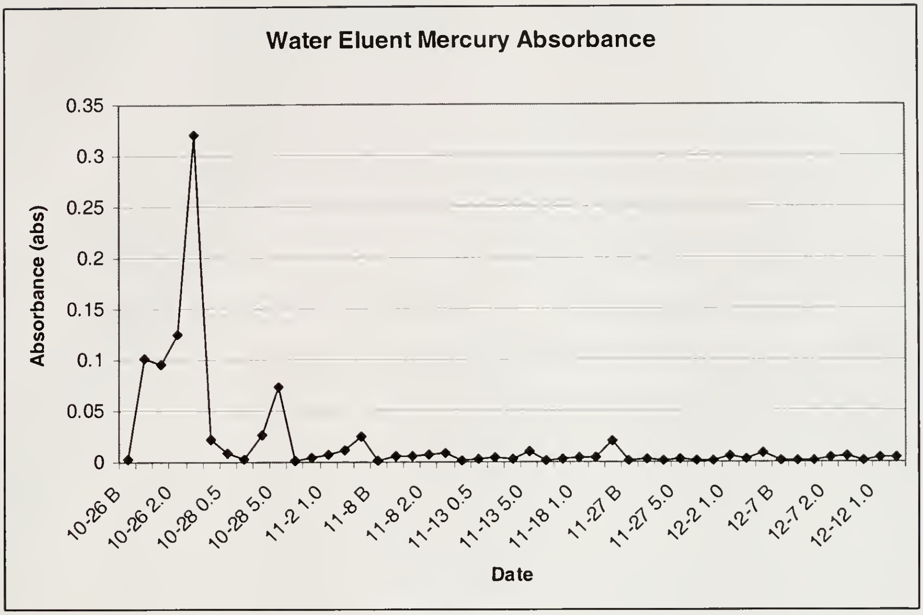


Figure 14: The absorbance data from the AAS for the water eluent samples. Notice how as time increases, the absorbance reading decreases over time.

8270 6250 17
10/25/06 VA